Degradation of [^{14}C]Amidosulfuron in Aqueous Buffers and in an Acidic Soil

Allan E. Smith* and Andrew J. Aubin

Research Station, Agriculture Canada, Box 440, Regina, Saskatchewan S4P 3A2, Canada

The hydrolysis of the herbicide [^{14}C]amidosulfuron (HOE 075032) was investigated in buffered aqueous solutions with pH values of 5, 7, and 9 at temperatures of 10, 20, 30, 40, and 50 °C. Hydrolysis was both temperature and pH dependent following first-order kinetics. The half-lives ranged from 1.1 day in buffer at pH 5 and 50 °C to >500 days in solution of pH 9 at 10 °C. Activation energies for the hydrolysis of [^{14}C]amidosulfuron at pH 5, 7, and 9 were 101, 121, and 135 kJ/mol, respectively. Under all conditions [^{14}C]-2-amino-4,6-dimethoxypyrimidine was the major degradation product, though a transient ^{14}C degradation product was formed in all solutions at pH 5. Incubation of [^{14}C]amidosulfuron in a silt loam (pH 5.2) at 20 °C and 85% field capacity (21% moisture) resulted in herbicide degradation that followed first-order kinetics with a half-life of 36 ± 6 days. [^{14}C]-2-Amino-4,6-dimethoxypyrimidine did not appear to be a degradation product in the silt loam. Solvent-nonextractable radioactivity associated with the silt loam after 12 weeks of incubation accounted for approximately 33% of the applied ^{14}C.

INTRODUCTION

Sulfonylurea herbicides are applied as postemergence treatments of wettable powders or water-dispersible granules at rates of between 10 and 20 g (ai)/ha for the postemergence control of broadleaf weeds and certain grasses in cereals (Beyer et al., 1988; Blair and Martin, 1988; Brown, 1990).

These compounds are nonvolatile and possess an ionizable proton on the amido group adjacent to the sulfonyl group, thus behaving as weak acids with pK_a values in the range 3–5 (Beyer et al., 1988; Brown, 1990; Hay, 1990). In the ionized state, their water solubilities are greater than in the neutral form (Beyer et al., 1988; Brown, 1990; Hay, 1990). Soil adsorption is, in general, low (Harvey et al., 1985; Mersie and Foy, 1985; Walker and Welch, 1989; Walker et al., 1989), and thus their leaching potential in alkaline field soils is high.

Sulfonylureas undergo hydrolysis in aqueous media at a rate dependent upon temperature and pH (Beyer et al., 1988; Brown, 1990; Hay, 1990; Sabadie, 1990, 1991). Under hydrolytic conditions, cleavage of the sulfonylurea bridge occurs with formation of a sulfonamide and an aminotriazine or aminopyrimidine (Beyer et al., 1988; Brown, 1990; Hay, 1990). An additional hydrolytic pathway involving the conversion of the methoxy group on the triazine ring of chlorsulfuron and metsulfuron methyl to a hydroxyl, prior to bridge cleavage, has been reported (Sabadie, 1990, 1991). Further hydrolysis products of the breakdown products may also occur (Beyer et al., 1988; Sabadie, 1990, 1991).

Sulfonylurea herbicides are degraded in the soil as a result of both biological and chemical mechanisms, with the latter being particularly important in acidic soils (Joshi et al., 1985; Beyer et al., 1988; Brown, 1990; Cambon et al., 1992). Since residues of these herbicides can exhibit activity in the soil for more than a year (Beyer et al., 1988; Blair and Martin, 1990; Hay, 1990), factors affecting their breakdown are of importance.

Amidosulfuron (Figure 1, 1), also referred to as HOE 075032 [3-(4,6-dimethoxypyrimidin-2-yl)-1-[*N*-methyl-*N*-(methylsulfonyl)amino]sulfonylurea], is currently being evaluated for the postemergence control of broadleaf weeds in cereal and other crops. Under laboratory conditions in three soils with pH values between 7.3 and 7.6, degradation rates were dependent upon both temperature and soil moisture with half-life values (time for degradation of 50% of the applied herbicide) ranging from 14 days in a sandy loam incubated at 30 °C to 231 days in a clay at 10 °C (Smith and Aubin, 1992).

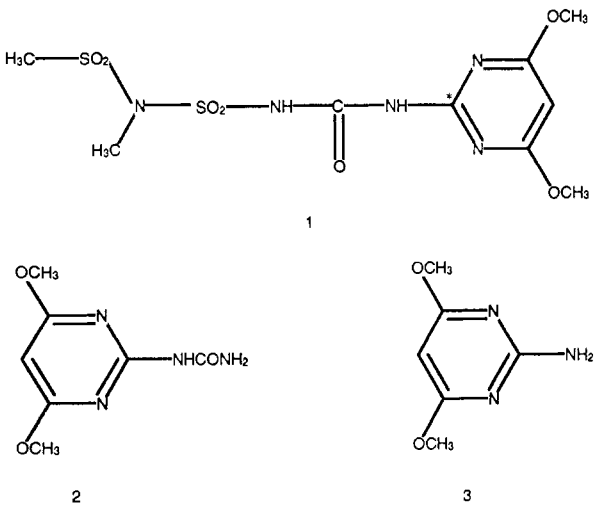

Figure 1. Structures of amidosulfuron (1, the asterisk denotes the position of the ^{14}C label), 4,6-dimethoxypyrimidin-2-ylurea (2), and 2-amino-4,6-dimethoxypyrimidine (2-ADMP, 3).

The following studies were undertaken to determine the rates of hydrolytic breakdown of [^{14}C]amidosulfuron in aqueous buffer solutions (pH 5.0, 7.0, and 9.0) at 10, 20, 30, 40, and 50 °C and to compare them with the previously determined soil degradation rates and with that in an acidic soil (pH 5.2) at 20 °C and 21% soil moisture.

MATERIALS AND METHODS

Soil. The silt loam was collected from the 0–5-cm soil horizon at a location near Leland, MS, in June 1992 and shipped to Regina. On arrival, the soil, which had a 15% moisture content, was sieved using a 2-mm screen and stored in a plastic sack at 4 ± 1 °C until October 1992, when the study was initiated. Soil texture analysis (Saskatchewan Soil Testing Laboratory, Saskatoon, SK) gave clay, sand, and silt contents of 18%, 15%, and 67%, respectively;

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an organic matter content of 1.2%; and a pH of 5.2. Field capacity, the percent moisture at 5-kPa suction, was 25%.

Chemicals. 3-(4,6-Dimethoxypyrimidin-[2-^{14}C]-yl)-1-[*N*-methyl-*N*-(methylsulfonyl)amino]sulfonylurea was obtained from Hoechst Aktiengesellschaft (Frankfurt am Main, Germany) with a specific activity of 2.57 MBq/mg and a radiochemical purity >96%. The [^{14}C]amidosulfuron was dissolved in 95% ethanol to give a solution with 550 kBq/mL and containing 214 μg of amidosulfuron/mL. Analytical samples (>98% pure) of nonradioactive amidosulfuron and 2-amino-4,6-dimethoxypyrimidine (2-ADMP; Figure 1, 3) were obtained from Hoechst Aktiengesellschaft and Aldrich Chemical Co. (Milwaukee, WI), respectively. For thin-layer chromatographic (TLC) purposes, a single methanolic solution was prepared containing 2 mg/mL of each chemical.

Hydrolysis of [^{14}C]Amidosulfuron. Buffered solutions at pH 5.0 and 7.0 were prepared by the addition of the appropriate amounts of 0.2 M disodium hydrogen phosphate to 0.1 M citric acid and buffer at pH 9.0 by adding 0.05 M sodium tetraborate to 0.1 M boric acid. To 12.0 mL of each buffer solution was added 350 μL of [^{14}C]amidosulfuron to give solutions containing 16.0 kBq and 6.22 μg of herbicide per mL. Aliquots (1.0 mL) of all three buffer solutions, containing radioactive herbicide, were added to each of 10 1.75-mL GC autosample vials. The vials were fitted with crimp caps to prevent evaporation and duplicate vials stored in light-tight metal containers at 10 ± 1, 20 ± 1, 30 ± 1, 40 ± 1, and 50 ± 1 °C. At appropriate time intervals, varying from daily for the studies conducted at 50 °C to monthly for those at 10 °C, 10-μL aliquots were removed from each vial and applied to the origins of TLC plates coated with 0.25-mm layers of silica gel 60F-254 (E. Merck, Darmstadt, Germany). At every sampling the vials received new crimp caps. The TLC plates were developed to a height of 10 cm above the origin using a mixture of ethyl acetate, toluene, and acetic acid (10:10:1) as developing solvent. After air-drying, the chromatograms were examined quantitatively for radioactive products using a Berthold automatic TLC linear analyzer (Labserco Ltd., Oakville, ON). Nonradioactive amidosulfuron and 2-ADMP were run for comparative purposes and identified by viewing the developed chromatograms under a shortwave ultraviolet lamp. The R_f values of 2-ADMP and amidosulfuron were 0.55 and 0.70, respectively. At the conclusion of each temperature study, the radioactivity in a portion (10 μL) of the remaining solution was determined. This confirmed there had been no loss of radioactivity during incubation. The experiment was terminated after 126 days.

Confirmation of 2-ADMP as a Hydrolysis Product. Aqueous extracts (250 μL) were evaporated to dryness in a 15-mL tapered glass tube at 40 °C using a stream of nitrogen, and the residue was dissolved in acetone (250 μL). Aliquots (2 μL) were injected into a Hewlett-Packard 5890A gas chromatograph equipped with a 5970 mass ion detector and scanned from 10 to 200 amu. The column was of fused silica (25 m × 0.25 mm) coated with 0.33 μm of HP-1 Ultra (Hewlett-Packard Ltd.). Carrier gas was helium with a gas linear velocity of 25.3 cm/s. Initial column temperature was held at 70 °C for 1 min after injection and then increased at a rate of 5 °C/min to a final temperature of 250 °C. The mass spectrum of the product in the acetone extracts with the same retention time (16.53 min) as authentic 2-ADMP was identical to that of the latter with major ions at *m/e* 155, 140, 125, and 68.

Soil Degradation Studies. The experimental design was the same as that recently described for similar laboratory persistence studies with [^{14}C]amidosulfuron in Saskatchewan soils (Smith and Aubin, 1992). Samples (50 g) of the silt loam at 21% moisture (85% of field capacity) were incubated, in polystyrene foam cartons with plastic lids, in the dark at 20 ± 1 °C for 7 days and then treated with [^{14}C]amidosulfuron (30 μL, 16.5 kBq, 6.42 μg of herbicide). The amidosulfuron concentration of 0.13 μg/g of moist soil was slightly higher than the 0.1 μg/g used in the previous study (Smith and Aubin, 1992). After thorough mixing with a spatula, the cartons were capped and reincubated at 20 ± 1 °C. Water was added, by weight and with stirring, as necessary to replace moisture lost by evaporation. Triplicate samples were extracted and analyzed 15 min and 2, 4, 8, and 12 weeks after treatment. For control purposes, to assess nonbiological degradation, air-dry soil samples (4% moisture, 16% FC) were similarly fortified with the ^{14}C-labeled herbicide and incubated for 12 weeks prior to extraction and analysis.

Table I. Hydrolytic Half-Lives of [^{14}C]Amidosulfuron in Buffered Solutions with Temperature

	half-life[a] (days)		
temp (°C)	pH 5	pH 7	pH 9
10	208	>500	>500
20	61	>500	>500
30	13	186	>200
40	4.1	42	46
50	1.1	9.6	10.2

[a] Mean from duplicate experiments.

Soil Extraction and Analysis. The extraction and analysis were identical to that previously described (Smith and Aubin, 1992). Thus, the soils were shaken with aqueous acidic acetonitrile, and following centrifugation, the solvent-extractable radioactivity was measured. After partitioning of extract between 5% aqueous sodium chloride and dichloromethane, radioactivity in the aqueous solution was measured, the organic phase evaporated to dryness, and the residue dissolved in methanol. Radioactivity in the methanolic solution was determined and the remaining solution evaporated with a stream of dry nitrogen to <0.5 mL. These evaporated extracts were then examined by TLC and ^{14}C products quantified by radiochemical analysis. After solvent extraction, the soil was collected by vacuum filtration, washed, and dried, and triplicate samples were assayed for radioactivity by combustion analysis, as described (Smith and Aubin, 1992).

Measurement of Radioactivity. Radioactivity in the various solutions was determined using a Packard Tri-Carb 1900 TR liquid scintillation analyzer. Scinti-Verse II (15 mL, Fisher Scientific Co., Fair Lawn, NJ) was used as scintillation solution, and counting efficiency corrections were applied using a ^{133}Ba external standard. Radioactivity associated with the solvent-extracted soils was determined by combustion analysis of samples (1 g) in a Harvey biological oxidizer, Model OX500 (R. J. Harvey Instrument Corp., Hillsdale, NJ). Recoveries of ^{14}C as [^{14}C]carbon dioxide from silt loam fortified with known amounts of [^{14}C]-amidosulfuron, immediately prior to combustion, were greater than 98%.

RESULTS AND DISCUSSION

In all three buffer solutions, [^{14}C]amidosulfuron underwent hydrolysis to [^{14}C]-2-ADMP, whose structure (Figure 1, 3) was confirmed by GC/MS analysis. 2-ADMP was stable in solution at all pH values and temperatures studied. At all samplings, there was very close agreement (<5% difference) in the data from the duplicate samples. For experiments conducted at 10 and 20 °C in buffers at pH values of 7 and 9, hydrolysis of [^{14}C]amidosulfuron was slow and the half-life values were estimated by extrapolation. At all other temperatures and pHs, herbicide dissipation followed first-order kinetics (R^2 values >0.91). The half-life values are compared in Table I. The rate of hydrolysis was dependent upon temperature and solution pH as has been noted for similar studies with other sulfonylurea herbicides (Beyer et al., 1988; Brown, 1990; Hay, 1990; Sabadie, 1990, 1991; Cambon et al., 1992). From the kinetic data (Table I) at 20 (pH 5 only), 30, 40, and 50 °C, the activation energies for hydrolysis of [^{14}C]-amidosulfuron at pH 5, 7, and 9 were calculated from the Arrhenius equation (Sabadie, 1991) as 101 ± 4, 121 ± 3, and 135 ± 10 kJ/mol, respectively (Table II). Such activation energies are similar to those reported for chlorsulfuron, chlorimuron ethyl, metsulfuron methyl, and sulfometuron methyl (Table II). These energies range from 83 to 135 kJ/mol, but in the absence of published statistical data it is impossible to draw conclusions as to

Table II. Activation Energies for the Hydrolysis of Sulfonylurea Herbicides in Buffered Solutions as a Function of pH

sulfonylurea	pH	activn energy (kJ/mol)	ref
amidosulfuron	5	101 ± 4	present study
	7	121 ± 3	
	9	135 ± 10	
chlorimuron ethyl	5	103	calculated from data of Beyer et al. (1988)
	6	117	
	7	127	
	8	129	
chlorsulfuron	4	92	Sabadie (1991)
	5	103	calculated from data of Beyer et al. (1988)
	6	108	
	7	134	
	8	118	
metsulfuron methyl	4	84	Sabadie (1990)
	5	104	calculated from data of Beyer et al. (1988)
sulfometuron methyl	6.4	117 ± 4	Cambon et al. (1992)
	5	120	calculated from data of Beyer et al. (1988)
	6	125	
	7	83	
	8	95	

whether there are significant differences between hydrolytic activation energies with solution pH or activation energies with sulfonylurea structure.

Solution pH controls the rate of hydrolysis since the neutral form of the sulfonylurea bridge is considerably more susceptible to hydrolysis than the ionic form (Brown, 1990; Hay, 1990). With a pK_a of 3.58, amidosulfuron is >99% ionized at pH >5.6, whereas at pH 5 about 5% of the molecule exists in an un-ionized form. At all temperatures, the rates of hydrolysis of amidosulfuron (Table I) at pH 7 and 9 are similar and considerably slower than in solution at pH 5. In general, the rates of hydrolysis at the three pH values resemble those of chlorsulfuron and metsulfuron methyl, being slower than the solution hydrolysis of chlorimuron ethyl, and sulfometuron methyl in the pH range 5–9 (Beyer et al., 1988; Sabadie, 1990, 1991; Cambon et al., 1992).

At all temperatures in solution at pH 7 and 9, over 93% of the radioactivity was identified by TLC analyses as either [^{14}C]amidosulfuron or [^{14}C]-2-ADMP. In contrast, all solutions at pH 5 revealed the additional presence of a ^{14}C polar product (R_f 0.05) whose concentration increased with time before being itself hydrolyzed. The data for the 50 °C study are shown (Figure 2) and indicate that a maximum of about 25% of the applied radioactivity was in such a form after 3 days. [^{14}C]-2-ADMP (Figure 1, 3) was the only other degradation product, and it was therefore concluded that, after formation, the unknown polar product was itself being hydrolyzed at pH 5 to 2-ADMP. From these data, the unknown product was tentatively considered to be the substituted pyrimidine–urea (Figure 1, 2). Thus, it would appear that the hydrolysis of amidosulfuron in acidic solution is different from that in and neutral solution as suggested (Brown, 1990; Hay, 1990).

It has been reported that both chlorsulfuron and metsulfuron methyl in buffer at pH 4 are hydrolyzed at the methoxy group of the triazine ring system with formation of a hydroxyl function (Sabadie, 1990, 1991).

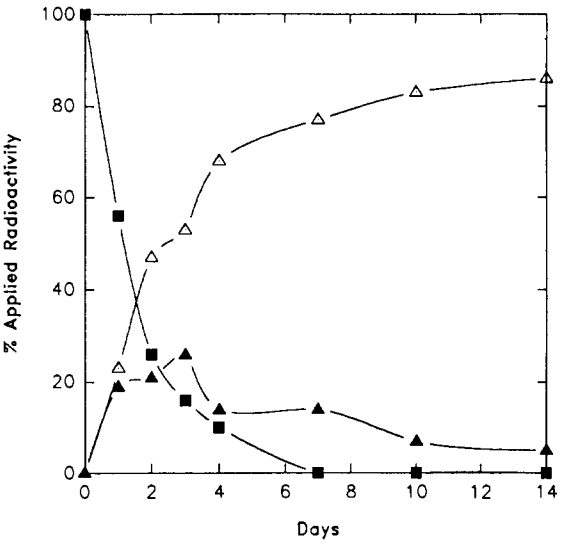

Figure 2. Percent of applied radioactivity identified as amidosulfuron (■), 2-amino-4,6-dimethoxypyrimidine (Δ), and unidentified product (▲) with time during the hydrolysis of [^{14}C]amidosulfuron in pH 5 buffer solution at 50 °C. Each data point is the average of two replicates.

These intermediates then undergo further hydrolysis at the sulfonylurea bridge, as well as cleavage of the triazine ring. In the present study there was no evidence of any such demethylation reactions involving the pyrimidine ring of [^{14}C]amidosulfuron.

The results of the persistence study conducted with [^{14}C]-amidosulfuron in the silt loam at 21% moisture (85% field capacity) and 20 °C are summarized in Table III. At all sampling times, solvent-extractable radioactivity together with that associated with the soil in a nonextractable form was >94% of that applied. Following partitioning of the acetonitrile soil extracts between aqueous sodium chloride and methylene chloride, evaporation of the latter, and the taking up of the residue in methanol, TLC and radiochemical analysis indicated [^{14}C]amidosulfuron to be the major component (Table III). Small amounts of other ^{14}C products were observed but in amounts too small to allow further characterization. It was observed (Table III) that the amounts of combined radioactivity in the methanolic and aqueous extracts were sometimes less than the total solvent-extracted ^{14}C. As before (Smith and Aubin, 1992), such differences were attributed to possible losses of volatile products on evaporation of the methylene chloride extracts and the inability of methanol to dissolve certain ^{14}C degradation products from the residue adhering to the glass of the evaporation flask.

With time, there was a loss of the ^{14}C-labeled herbicide that followed first-order kinetics (R^2 = 0.98). From these data (Table III), the half-life of [^{14}C]amidosulfuron was calculated as 36 ± 6 days. Under the same moisture and temperature conditions, and with the same experimental procedure, the half-lives for [^{14}C]amidosulfuron in a loamy sand (pH 7.3) and a clay (pH 7.6) were 26 ± 3 and 45 ± 11 days, respectively, and in a clay of pH 7.5, 79 ± 2 days (Smith and Aubin, 1992). In all cases, the rate of breakdown of amidosulfuron in the soils was more rapid than would be expected by simple solution hydrolysis (cf. Table I). The greater acidity of the silt loam (pH 5.2) did not appear to result in a greater increase in the rate of degradation of amidosulfuron compared to alkaline soils. Some of this difference must be attributed to biological processes with presumably fewer amidosulfuron-degrading organisms in the silt loam, used in the present study, than

Table III. Radioactivity Recovered with Time from a Silt Loam at 85% Field Capacity Treated with 0.13 μg/g [14C]Amidosulfuron following Incubation at 20 °C

14C extracted from soil	% of applied radioactivity[a]				
	after 0 weeks	after 2 weeks	after 4 weeks	after 8 weeks	after 12 weeks
solvent extractable	102 ± 1	90 ± 1	83 ± 0	71 ± 1	61 ± 1 (83 ± 1)[b]
methanol soluble	101 ● 4	76 ± 2	63 ± 1	45 ± 2	36 ± 2 (61 ± 3)
aqueous soluble	1 ± 1	4 ± 1	5 ± 1	25 ± 1	27 ± 1 (20 ± 1)
amidosulfuron	94 ± 6	68 ± 3	55 ● 4	37 ± 7	24 ± 3 (51 ± 6)
soil associated[c]	4 ± 1	12 ± 1	18 ± 1	27 ± 1	33 ± 2 (13 ± 1)
total 14C recovered	106 ± 1	102 ± 2	101 ± 1	98 ± 1	94 ● 4 (96 ● 1)

[a] Mean and standard deviation from three replicates. [b] Figures in parentheses represent data obtained from air-dry soils (4% moisture, 16% FC). [c] Determined by combustion of the solvent-extracted soils.

in the two Saskatchewan soils. Other studies note that the breakdown of chlorsulfuron and metsulfuron methyl was faster in acidic soils than in neutral (Thirunarayanan et al., 1985; Beyer et al., 1987; Walker et al., 1989).

In the air-dry silt loam (4% moisture, 16% field capacity) some degradation of [14C]amidosulfuron occurred (Table III) with 51% being recoverable after 12 weeks. This breakdown is greater than that (14%) noted in an air-dry (<10% field capacity) loamy sand under similar conditions (Smith and Aubin, 1992). Although the moisture level of the silt loam was low, it is still possible that some biological breakdown had occurred. However, chemical degradation is also possible. Both chlorsulfuron and metsulfuron methyl undergo extensive breakdown on dry mineral supports at room temperature (Sabadie and Bastide, 1990; Sabadie, 1992).

Since 2-ADMP is a major hydrolysis product of amidosulfuron in aqueous solutions, it was considered as a possible soil degradation product. Prior studies indicated that the recovery of radioactivity from triplicate samples of silt loam (10 g) treated with [14C]-2-ADMP (250 μL, 0.65 μg, obtained by hydrolysis of [14C]amidosulfuron in buffer solution at pH 5 at 50 °C for 14 days) with 20 mL extractant, as described, was 84 ± 10%. Over 95% of the recovered radioactivity was partitionable as [14C]-2-ADMP into dichloromethane. Thus, if this hydrolytic product were formed during soil incubation, it would be detectable in the methanolic extracts. At all samplings, if [14C]-2-ADMP was isolated, it accounted for <5% of the applied radioactivity and was not positively identified. However, it is possible 2-ADMP could be formed transiently before undergoing biological modification to demethylated hydroxypyrimidines.

As in the earlier study (Smith and Aubin, 1992), with time a considerable amount of the applied radioactivity was converted into polar water-soluble and dichloromethane-insoluble degradation products (Table III) with probable structures based on those of biologically modified pyrimidines. No attempts were made to characterize such products. At all sampling dates there were greater amounts of soil-associated solvent-nonextractable radioactivity than those levels encountered with the three soils of the previous study (Smith and Aubin, 1992). After 12 weeks, approximately 33% (Table III) of the applied radioactivity remained in the soil following solvent extraction, compared to the 7–11% observed earlier (Smith and Aubin, 1992). The nature of this solvent-nonextractable radioactivity is unknown but could include both 14C material incorporated into the microbial biomass and soil organic matter and 14C degradation products not recoverable using the extraction procedure (Smith and Aubin, 1992).

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